Journal of Agricultural Science (2008), **146**, 667–675. © 2008 *Cambridge University Press*
doi:10.1017/S0021859608007971 *Printed in the United Kingdom*

Soybean seed composition in cultivars differing in resistance to charcoal rot (*Macrophomina phaseolina*)*

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(*Revised MS received 2 April 2008; First published online 22 August 2008*)

SUMMARY

Seed composition in soybean [*Glycine max* (L) Merr.] has not been well investigated under charcoal rot infestation under irrigated and non-irrigated conditions. The objective of the present experiment was to assess seed composition and nitrogen fixation under these conditions. No significant differences in protein levels in the moderately resistant germplasm line DT97-4290 were observed under these conditions. Under irrigation, protein concentration was significantly ($P \leqslant 0{\cdot}05$) higher for the susceptible cultivars Egyptian and Pharaoh under non-infested than infested conditions. The opposite response for protein was observed under non-irrigated conditions for Pharaoh. Oleic acid concentration was significantly ($P \leqslant 0{\cdot}001$) higher in susceptible cultivars under infested conditions. The concentration of linolenic acid in susceptible cultivars was significantly lower under infested conditions. The enrichment of Delta ^{15}N in susceptible cultivars under infested conditions indicated that nitrogen fixation was substantially inhibited, but soil nitrogen was used for compensating for atmospheric nitrogen inhibition. These results indicate that charcoal rot infection may alter seed composition and nitrogen fixation in soybean. The alteration in seed composition depended on cultivar susceptibility to charcoal rot and irrigation management.

INTRODUCTION

Soybean is produced mainly for protein and oil (seed composition). Information on the effects of charcoal rot disease on soybean seed composition under the Early Soybean Production System (ESPS; Heatherly *et al.* 1998) in mid-southern USA, including Mississippi, Louisiana, Alabama, Tennessee, and Kentucky, is limited. Charcoal rot is caused by the fungus *Macrophomina phaseolina*. The pathogen can be found worldwide and is most severe between 35°N and 35°S (Wyllie 1976) under warm temperatures and drought (Smith & Wyllie 1999), with an optimum temperature range 30–35 °C for disease expression (Meyer *et al.* 1974). The pathogen affects soybean plants by reducing root volume and weight and also plant height, leading to a significant negative effect on yield (Wrather *et al.* 1995) and seed quality (Gangopadhyay *et al.* 1970).

Although tremendous efforts are being made to identify charcoal rot resistance in soybean, no claims have been made for soybean charcoal rot resistance in commercial cultivars. Although it has been reported that irrigation is recommended to help alleviate stress, recent work (Kendig *et al.* 2000) showed that water management limits, but does not prevent root infection. A moderately resistant germplasm line, DT97-4290, was field evaluated and released (Paris *et al.* 2006) at Stoneville, MS, USA. Host tissue colonization by *M. phaseolina* was assessed (Mengistu *et al.* 2007). Colony forming units (Smith & Carvil 1997) of *M. phaseolina* were also used as a measure of resistance.

* Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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Since the use of ESPS in the midsouth, especially in Mississippi, has exposed ESPS cultivars to new environmental stress factors during seed fill and since there is limited information on the effects of charcoal rot infection on seed composition, the objective of the present experiment was to investigate the concentration of protein, oil and fatty acids as influenced by cultivar susceptibility to charcoal rot grown under charcoal rot infestation and under irrigated and non-irrigated conditions. To study the relationship between protein concentration and nitrogen fixation under those conditions, nitrogen fixation using nitrogen isotope $^{14}N/^{15}N$ was also assessed.

MATERIALS AND METHODS

Selected soybean cultivars of maturity group (MG) IV were planted in sandy loam soil in 2004 and 2005 at Delta Research and Extension Center Stoneville, MS (33°26′N). Cultivars were selected based on their susceptibility to charcoal rot disease as follows: (a) moderately resistant germplasm line DT97-4290 and (b) susceptible cultivars Egyptian and Pharaoh. Seeds were treated with mefenoxam ((R)-2-{2, 6-(dimethylphenyl)-methoxyacetylamino}-propionic acid methyl ester) fungicide prior to planting as a precaution against stand loss due to *Pythium* spp. Two weeks prior to planting, individual plots were fumigated using a mix of Methyl Bromide in combination with chloropicrin in the proportions 0·895 and 0·105, respectively, at a depth of 254 mm at a broadcast rate of 504 kg/ha. The plots were then either infested with charcoal rot inoculum, or not (non-infested). Row spacing was 0·5 m and seeding rate was 16–20 seeds/m of row. Plots were 23 m long. In all years plots were 4 m (eight rows) wide. Planting was complete by the end of April in 2004 and 2005 and plants were harvested in mid-September. Throughout the present study, C = control or non-infested; I = irrigated; N = non-irrigated; and D = infested or diseased. Therefore, the treatments were irrigated/non-infested (IC) as a control for irrigated/diseased (ID); non-irrigated/non-infested (NC) as a control for the non-irrigated/infested (ND). We used infestation/infested or infection/infected based on the definition of Holliday (1989). Therefore, the term 'infestation/infested' has been used whenever charcoal rot conditions in soil or charcoal rot treatment are referred to; 'infection/infected' has been used whenever the reference is to charcoal rot in relation to plant, cultivar, or seed. Irrigation was applied via furrows whenever water potential reached −50 KPa at 300 mm depth and soil water potential was monitored using tensiometers according to Heatherly *et al.* (2001), Ray *et al.* (2006) and Bellaloui & Mengistu (2008). Irrigation was applied to designated plots beginning from emergence (VC) to the end of seed fill (R6) growth stage (Fehr *et al.* 1971). Inoculum increase, inoculation and disease assessments were based on the method developed by Mengistu *et al.* (2007). Infected millet seeds were used to inoculate plots at the rate of 3 g/m of a row at planting. Disease was measured by the intensity of discolouration on a scale of 1–5, where 1 = no discolouration and 5 = highly discoloured. The scale for root and stem severity (RSS) was divided into four classifications (Paris *et al.* 2006): resistant (values of 1), moderately resistant (values >1 and $\leqslant 2$), moderately susceptible (values >2 and <3) and susceptible (values 3–5). Host tissue colonization by *M. phaseolina* was assessed in three replications by destructively sampling 10 plants at the R7 growth stage. Soybean was harvested from each treatment and grain yield was adjusted to 130 g moisture/kg.

Analysis of protein, oil and fatty acids in seed

Seeds (approximately 25 g of whole grain) from each replicate were analysed for oil, fatty acids and protein using near infrared (NIR; Wilcox & Shibles 2001; Dardanelli *et al.* 2006) reflectance (Diode array feed analyser, Perten). The results were expressed as a concentration on a dry matter basis (Wilcox & Shibles 2001).

Delta ^{15}N natural abundance analysis

Delta ^{15}N abundance was evaluated from nitrogen isotope $^{15}N/^{14}N$ ratio (Shearer & Kohl 1986) as described by Bellaloui *et al.* (2008).

Experimental design and statistical analysis

The experimental design was a split plot with irrigation as the main plot, infestation as subplot, and cultivar as sub-subplot. Three replicates were used. Differences in means were considered significant at $P \leqslant 0{\cdot}05$. Results were analysed by SAS, using Proc Mixed procedure (SAS 2001).

RESULTS

Effects of charcoal rot infection on seed oil, fatty acids and protein

Table 1 shows the score of mean severity of charcoal rot across 2 years on the three cultivars tested. In the control plants, there were no significant ($P \leqslant 0{\cdot}05$) differences between the varieties for charcoal rot score. Under infested conditions, Pharaoh and Egyptian had a significantly higher ($P \leqslant 0{\cdot}05$) score than DT97-4290. Analysis of variance across years showed significant ($P \leqslant 0{\cdot}01$) effects of year, irrigation and cultivar for protein, oil and fatty acid oleic. There were significant year × cultivar and year × infestation interactions for oleic ($P \leqslant 0{\cdot}01$) and linolenic

Table1. Score *of mean severity of charcoal rot infection across years in cultivars under irrigated-infested/diseased* (*ID*), *non-irrigated-infested/diseased* (*ND*), *irrigated-non-infetsed/control* (*IC*), *and non-irrigated-non-infested/control* (*NC*) *conditions**

	Under infested conditions		Under non-infested conditions	
Cultivar	ID	ND	IC	NC
Pharaoh	2·35	3·05	1·45	1·90
Egyptian	2·15	2·95	1·65	1·30
DT 97-4290	1·30	1·20	1·20	1·15
S.E.D.† (D.F. = 15)	0·231	0·353	0·151	0·442

* The severity of internal discolouration for stem and root was measured on a scale of 1–5 (where 1 = resistant and 5 = susceptible) according to Paris *et al.* (2006). A rating value of 1 = resistant, > 1 and ⩽ 2 = moderately resistant, > 2 and < 3 = moderately susceptible, and 3–5 = susceptible.
† S.E.D. = Standard error of differences.

($P \leqslant 0{\cdot}05$) acids, as well as significant cultivar × infestation interactions for protein ($P \leqslant 0{\cdot}001$) and fatty acids ($P \leqslant 0{\cdot}001$). Since there were year × cultivar and year × infestation interactions for some seed composition components (protein and fatty acids), the results are presented separately for each year.

Protein and oil content in 2004 and 2005 are presented in Table 2. In 2004 and 2005, no significant differences in protein or oil levels in DT97-4290 were observed. However, in the cultivar Egyptian, protein was significantly higher under IC than under ID conditions for 2 years. The opposite was observed when Egyptian was grown under non-irrigated conditions, where protein was consistently higher under ND than NC in both years. Pharaoh showed a different pattern in that an increase in protein concentration was observed under non-infested conditions (IC and NC). Oil (Table 2) and saturated fatty acids (palmitic and stearic, data not shown) did not show a consistent pattern.

No effects of infestation on oleic and linolenic acid concentrations in DT97-4290 were observed. In the susceptible cultivars, however, oleic acid was significantly ($P \leqslant 0{\cdot}0001$) higher in Egyptian under ID than IC for 2 years (Table 3). For Pharaoh, oleic acid was significantly higher under infested (ID and ND) conditions for 2 years (Table 3). It is interesting to note that oleic acid was consistently higher under infested conditions than non-infested conditions in Pharaoh.

Linolenic acid had the opposite trend to oleic acid and was significantly lower under infested conditions in both years in both susceptible cultivars (Table 3).

The effect of irrigation type (irrigated and non-irrigated) on protein, oil and oleic and linolenic acids was inconsistent across years (Tables 4 and 5). Yield was consistently higher under irrigated than under non-irrigated conditions in all cultivars (Table 4). However, no significant differences in yield were observed between infested and non-infested plots for each cultivar (Table 2).

Nitrogen fixation as influenced by charcoal rot

In both years there was a consistent significant ($P \leqslant 0{\cdot}05$) enrichment of Delta ^{15}N in susceptible varieties Egyptian and Pharaoh, especially under ND ($P \leqslant 0{\cdot}0001$; Fig. 1*c–f*). The lower enrichment of Delta ^{15}N in plants in non-infested plots of DT97-4290 indicated that atmospheric nitrogen (N_2) was the main source of nitrogen for nodules.

DISCUSSION

Source of variability

The main effect of year, irrigation, cultivar and infestation was the main source of seed composition variability. Interactions between these factors for some seed composition components indicate seasonal environmental factors of rainfall and temperature cannot be excluded as source of variability. It was observed that temperatures in 2004 were slightly higher than those in 2005 and precipitation during June was higher (10·5 mm in 2004 compared to 0·6 mm in 2005). The effect of environmental factors on soybean for seed yield, protein and oil has been reported to be significant (Wilcox & Shibles 2001). The weather data on rainfall and air temperature (Table 6) (MSUCares 2008) showed that the period from June to August is important, as it coincides with the reproductive period: initial bloom to full bloom (R1–R2) in June; beginning pod to full pod to beginning seed (R3–R4–R5) in July; and full seed to beginning maturity to full maturity (R6–R7–R8) in August. In mid-southern USA, the critical stages of development can occur during greatest drought stress in normal years (Heatherly *et al.* 1998). Therefore, environmental factors cannot be excluded as an additional possible source of seed composition variability. The effect of environmental factors on seed composition is beyond the scope of the present study.

Protein, oil and fatty acids

The stability of protein and oil contents in DT97-4290 and the change in protein and oil contents in susceptible cultivars may indicate that seed composition responds differently, depending on the susceptibility of the cultivar to charcoal rot infection. Although protein and oil levels in DT97-4290 did not decrease

Table 2. *Mean values of protein and oil concentrations (g/kg DM) in soybean as influenced by charcoal rot infection in 2004 and 2005*

Cultivar	Infestation treatment	2004 Protein	2004 Oil	2005 Protein	2005 Oil	2004/05 Yield (kg/ha)
DT97-4290	IC	370	210	401	186	4089
	ID	370	219	412	182	3597
	NC	357	224	398	172	2215
	ND	361	215	403	169	2762
	S.E.D.	4·4	4·0	7·2	3·9	291
	(D.F.)*	(6)	(6)	(6)	(6)	(18)
Egyptian	IC	415	210	440	175	3362
	ID	374	208	389	185	3129
	NC	368	200	398	174	2274
	ND	400	193	450	172	2329
	S.E.D.	7·5	3·3	4·6	3·1	184
	(D.F.)	(6)	(6)	(6)	(6)	(18)
Pharaoh	IC	404	214	450	188	4117
	ID	383	221	394	182	3906
	NC	392	212	428	177	2716
	ND	360	222	376	185	2984
	S.E.D.	5·4	5·3	7·4	3·5	303
	(D.F.)	(6)	(6)	(6)	(6)	(18)

* S.E.D. = Standard error of difference; D.F. = degrees of freedom.
Treatments are: IC = irrigated/non-infested; ID = irrigated/infested or irrigated/diseased; NC = non-irrigated/non-infested; ND = non-irrigated/infested or non-irrigated/diseased.

Table 3. *Mean values of oleic and linolenic acids concentrations (g/kg DM) in soybean as influenced by charcoal rot infection in 2004 and 2005*

Cultivar	Infestation treatment	2004 Oleic	2004 Linolenic	2005 Oleic	2005 Linolenic
DT97-4290	IC	229	63	145	129
	ID	219	66	164	110
	NC	236	61	187	118
	ND	237	64	200	108
	S.E.D. (D.F. = 6)*	7·6	2·0	9·7	5·2
Egyptian	IC	177	74	112	123
	ID	210	56	236	78
	NC	229	74	130	128
	ND	191	63	184	98
	S.E.D. (D.F. = 6)	7·4	2·7	13·0	7·1
Pharaoh	IC	203	72	101	130
	ID	244	51	155	93
	NC	186	75	141	117
	ND	230	61	181	94
	S.E.D. (D.F. = 6)	5·9	2·3	10·2	6·6

* S.E.D. = Standard error of difference; D.F. = degrees of freedom.

under charcoal rot infection, a significant ($P \leqslant 0·05$) decrease in isoflavones was observed in all cultivars under infection, regardless of the level of susceptibility to charcoal rot (data not shown). This indicates isoflavones in DT97-4290 are more sensitive to charcoal rot infection than protein and oil. The nature of this selective response is not known and requires further investigation.

The consistent decrease in protein and linolenic acid accumulation in Pharaoh under infection could be due to charcoal rot infection, leading to a reduction in seed quality (Gangopadhyay *et al.* 1970). Since there was a significant decrease in protein in Pharaoh under ND, non-irrigated conditions in combination with the infection may further decrease protein accumulation in this cultivar. This observation is supported by Kendig *et al.* (2000), who found that the severity of charcoal rot increased with drought conditions.

Charcoal rot infection did not always lead to a decrease in seed protein concentration as seen in Pharaoh under ID and ND. Egyptian showed a higher protein concentration under ND; this could be due to water stress and charcoal rot infection. Therefore, the trend of decrease or increase in seed protein in susceptible cultivars may depend on cultivar and irrigation management. This explanation is supported by other workers like Hoffman *et al.*

Table 4. *Mean values of protein and oil concentrations (g/kg DM) in soybean as influenced by irrigated (I) and non-irrigated (NI) conditions in 2004 and 2005*

Cultivar	Irrigation treatment	2004 Protein	2004 Oil	2005 Protein	2005 Oil	2004/05 Yield (kg/ha)
DT97-4290	I	370	215	407	184	3843
	N	359	219	400	170	2488
	S.E.D.	2·9	3·2	5·0	2·6	211
	(D.F.)*	(8)	(8)	(8)	(8)	(20)
Egyptian	I	394	209	424	180	3245
	N	384	197	414	173	2302
	S.E.D.	9·6	2·3	11·8	2·6	127
	(D.F.)	(8)	(8)	(8)	(8)	(20)
Pharaoh	I	393	218	422	185	4011
	N	376	217	402	181	2850
	S.E.D.	7·0	4·5	12·9	3·6	208
	(D.F.)	(8)	(8)	(8)	(8)	(20)

* S.E.D. = Standard error of difference; D.F. = degrees of freedom.

Table 5. *Mean values of oleic and linolenic acids concentrations (g/kg DM) in soybean irrigated (I) and non-irrigated (NI) conditions in 2004 and 2005*

Cultivar	Irrigation treatment	2004 Oleic	2004 Linolenic	2005 Oleic	2005 Linolenic
DT97-4290	I	224	65	154	119
	N	236	63	194	113
	S.E.D.	6·4	1·4	7·2	4·8
	(D.F. = 8)*				
Egyptian	I	193	65	174	101
	N	210	69	157	113
	S.E.D.	9·1	3·7	23	9·7
	(D.F. = 8)				
Pharaoh	I	224	61	128	112
	N	208	68	161	106
	S.E.D.	10·2	4·3	12·5	8·0
	(D.F. = 8)				

* S.E.D. = Standard error of difference; D.F. = degrees of freedom.

(1998), who showed that increasing infection with *S. sclerotiorum* led to increased seed protein content in cultivar A3304, but not in the other four cultivars. Ziems *et al.* (2007) found that infected soybean with bean pod mottle virus had increased seed protein and decreased oil. It has also been reported that the increase of protein concentration often reduces oil concentration (Brim & Burton 1979; Burton 1985; Wilson 2004) and often, but not always, lower yield (Brim & Burton 1979). The present results are in agreement with the above research for the protein increase in Egyptian in ND, for protein decrease in Pharaoh in ID and ND and for the inverse relationship between protein and oil concentrations (when protein and oil concentration in 2004 and 2005 are compared). Although the inverse relationship between protein and oil was thought to be genetically controlled, associated with environment and gene interactions and related to energy cost partitioning between protein and oil syntheses (Egli & Bruening 2007), the mechanism controlling this relationship is still unknown. It must be noted that the response of these cultivars to the infection and their impact on seed composition cannot be generalized for other susceptible or resistant cultivars. In addition, a combination of charcoal rot infection, genotype, environmental factors of rainfall and temperature could be another possible source of seed composition variability.

Other data from the present 2-year field experiment (not shown) suggested that the concentrations of calcium (Ca), zinc (Zn), and boron (B) in the seeds were significantly higher under irrigated and infected condition in DT97-4290 than in Egyptian and Pharaoh for the 2 years. Although the effect of mineral nutrition on fungal disease control has been reported extensively (Corden 1965; Ollagnier & Renard 1976; Graham & Webb 1991), the roles of Ca, B and Zn in the charcoal rot resistance and its influence on soybean seed composition is still not well known. One possible explanation is that Ca and B are taken up at higher rates in DT97-4290 than in Egyptian and Pharaoh as a response to disease infection for cell-wall stability and integrity. The roles of Ca and B in the cell wall has been reported by several authors (Konno *et al.* (1984) for Ca, and Brown & Hu (1996) and Hu *et al.* (1996) for B). The lower levels of Ca and

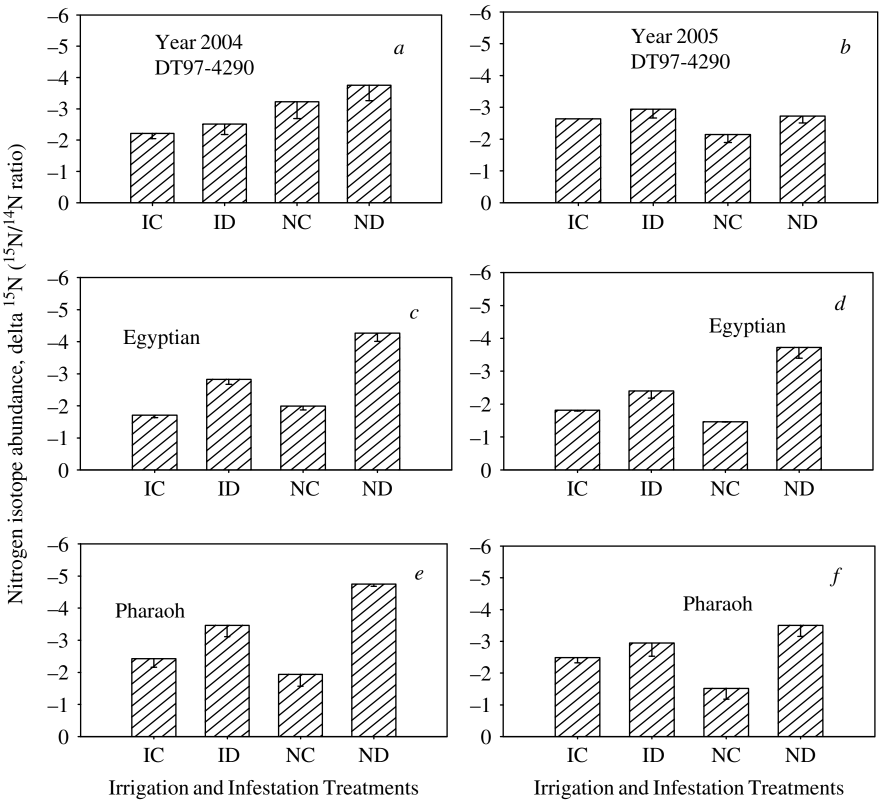

Fig. 1. The effect of irrigation and charcoal rot infection on nitrogen isotope abundance Delta ^{15}N as expressed by $^{15}N/^{14}N$ ratio in DT97-4290, Egyptian, and Pharaoh. Treatments are: IC = irrigated/non-infested; ID = irrigated/infested or irrigated/diseased; NC = non-irrigated/non-infested; ND = non-irrigated/infested or non-irrigated/diseased. Delta ^{15}N in 2004 (*a*, *c* and *e*); in 2005 (*b*, *d* and *f*). Values are mean of three replicates. Bars are mean ± standard error of the mean values (S.E.M.).

B in seed may be due to infected root in susceptible cultivars.

The consistent increase of oleic fatty acid in Pharaoh under infested conditions and in Egyptian under infested and irrigated conditions may suggest that oleic fatty acid may play a role under charcoal rot infection, or its increase may be a result of charcoal rot stress. The different trend between oleic and linolenic acid may be due to a reciprocal change in polyunsaturated fatty acids, which is related to changes in the activity of the desaturase enzyme (Bennett *et al.* 2004).

The effect of irrigation and non-irrigation on protein, oil, and oleic and linolenic acids was inconsistent across years. Kendig *et al.* (2000) showed that when irrigation was terminated in well-watered plots at growth stage R2, *Macrophomina phaseolina* microsclerotial densities in the roots increased, indicating increased root colonization. Matheny & Hunt (1981) and Sweeney *et al.* (2003) reported that protein and oil accumulation did not respond to irrigation, but Bellaloui & Mengistu (2008) found that irrigation type influenced significantly seed protein, oil, and oleic and linolenic acids in soybean. However, this influence depended on the cultivar. The inconsistency of the effect of irrigation on seed composition may be due to the variability in environmental factors such as rainfall and temperature during the growing season, and cultivar/genotype differences.

The significantly higher yield under irrigated than non-irrigated conditions in all the cultivars is in agreement with reports by Heatherly *et al.* (1999). In spite of the effect of charcoal rot infestation in soil on seed composition, no significant differences in yield between infested and non-infested plots were found for all cultivars within each irrigation treatment. It is clear that under infestation Egyptian and Pharaoh had a higher infection score than DT92-4290, but under non-infestation the endogenous pathogen was still present at lower levels. Therefore, a greater differential in charcoal rot pressure between the treatments may have been required to see an effect on yield. The authors of this paper suggest that

Table 6. *Air temperature (°C), rainfall (mm) at Delta Research and Extension Center, Stoneville, MS, USA in 2004, 2005, and their long-term monthly means and coefficient of variation (CV)*

	2004			2005			1996–2007		
Month	Tmax. (°C)* (CV)	Tmin. (°C)† (CV)	Prec. (mm)‡ (CV)	Tmax. (°C) (CV)	Tmin. (°C) (CV)	Prec. (mm) (CV)	Long-term mean Tmax. (CV)	Long-term mean Tmin. (CV)	Long-term mean Prec. (CV)
Apr	23·9 (21·7)	12·2 (41)	3·5 (243)	24·7 (17)	12·0 (27·6)	3·8 (264)	24·2 (7·3)	12·3 (15·3)	4·4 (42·7)
May	28·7 (13·3)	18·6 (20·8)	5·9 (171)	28·6 (16·6)	16·3 (26)	1·7 (279)	29·4 (4·0)	17·8 (7·5)	3·4 (48·8)
Jun	30·9 (7·7)	21·7 (9·0)	10·5 (190)	32·3 (9·3)	20·9 (9·2)	0·6 (473)	32·0 (3·0)	21·1 (3·6)	3·7 (71·7)
Jul	32·6 (8·5)	22·2 (8·2)	2·5 (283)	33·1 (7·9)	23·0 (6·2)	3·4 (173)	33·7 (2·8)	22·8 (2·8)	2·7 (59·4)
Aug	32·1 (8·0)	19·6 (17·4)	1·8 (303)	35·3 (6·6)	22·9 (6·1)	4·1 (332)	34·4 (5·5)	21·9 (4·8)	2·3 (86·4)
Sep	31·7 (6·3)	18·1 (17·7)	0·0 No rain	33·2 (9·4)	19·9 (13·9)	6·0 (515)	31·4 (4·1)	18·1 (8·2)	3·1 (60·1)

* Tmax. = maximum temperature.
† Tmin. = minimum temperature.
‡ Prec. = precipitation. CV was large because precipitation can range from 0 to170 mm in a month with only 1–3 times of rain per month; this is not unusual for mid-southern USA.

alteration in seed composition occurred in susceptible cultivars without yield differences. Although the alteration of seed composition can be explained as a stress response resulting from the charcoal rot infection, genotypic differences between cultivars and environmental factors of rainfall and temperature could be other possible sources for seed composition differences. The observation that seed composition changed without yield differences under stressed and non-stressed conditions was observed in soybean in an another study (Bellaloui *et al.* 2008). Bellaloui *et al.* (2008) found that the application of high dose of glyphosate to glyphosate-resistant soybean resulted in a significant increase in oleic acid and protein contents and decrease in linolenic acid in seed without yield differences between treated and non-treated soybean.

Nitrogen fixation using delta ^{15}N natural abundance analysis

The present results are in agreement with those reported by Sprent *et al.* (1996) in that plants which fix part or entire nitrogen content for their needs will have lower ^{15}N than plants which obtain entire nitrogen for their needs from the soil. The enrichment of ^{15}N under infested conditions in susceptible cultivars suggested charcoal rot may have negatively affected nodules' growth and nitrogen fixation capacity. One possible explanation is that when nitrogen fixation decreases, soil nitrate may be used to compensate for nitrogen fixation reduction caused by possible nodule damage in susceptible varieties. The higher ^{15}N/^{14}N ratio in the susceptible cultivars under infested conditions, especially under non-irrigated conditions indicates that both cultivars took up more ^{15}N (soil nitrogen) and less ^{14}N (atmospheric nitrogen), suggesting that nitrogen fixation was substantially lower under infested conditions and source of nitrogen used in nitrogen metabolism (assimilation and fixation) was altered. This suggestion is supported by the observation that the susceptible cultivars showed a significant alteration in ^{15}N/^{14}N ratio compared with the moderately resistant line. It appears that nitrogen fixation is more sensitive to infection than nitrogen assimilation. This may be one of the mechanisms used in maintaining nitrogen level in seeds under biological and environmental stresses (Bellaloui & Mengistu 2008), where nitrate uptake by roots and root nodules is inhibited. This observation may have a significant agricultural application for yield increase and higher seed quality through maintaining an optimum *Rhizobium* level under charcoal rot infection. The agricultural implication of this observation needs further investigation before a recommendation is made.

In conclusion, the increase or decrease of protein in susceptible cultivars in response to charcoal rot infection may depend on the cultivar and irrigation management. Maintaining protein concentrations in the moderately resistant line may be due to less infection by charcoal rot and healthy root system. The alteration in seed composition in susceptible cultivars could be due to the infection of the disease and root damage. The higher level of oleic acid under infected conditions is interesting and needs further investigation. Variability of seasonal factors of rainfall and temperature, and genotype differences between cultivars could be another possible source of seed compositional differences. The higher $^{15}N/^{14}N$ ratio in the susceptible cultivars under infected conditions may indicate the possible negative impact of charcoal infection on growth and function of nodule, and nitrogen fixation capacity. In spite of current management strategies to reduce the impact of charcoal rot on yield and seed quality, the most effective approach is the use of resistant or tolerant soybean cultivars. Since there are no commercial resistant cultivars available, and water management can have a significant effect on root colonization by *M. phaseolina* (Kendig *et al.* 2000), tolerant cultivars with appropriate water management may provide soybean producers with an alternative approach for charcoal rot management. The current information is valuable for soybean breeding programmes for seed composition improvement.

We are highly grateful to Dr Anne M. Gillen for reviewing the manuscript; Sandra Mosley, Gary Shelton, Will Marlow, Avis Clark, and Annie Johnson for field and laboratory technical assistance. Also, we are thankful to Dr Craig A. Abel and Leslie Price for their technical assistance in nitrogen isotope measurement.

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